

SEQUENCE LISTING

<110> Jung, Rudolf
Beach, Larry R.
Dress, Virginia M.
Rao, A. Gururaj
Ranch, Jerome P.
Ertl, David S.
Higgins, Regina K.



<120> Alteration of Amino Acid Compositions in
Seeds

<130> 0815A

<150> US 09/020,716

<151> 1998-02-09

<160> 22

<170> FastSEQ for Windows Version 3.0

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<211> 3363

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<223> pBSKP vector with native alpha hordothionin
sequence from Hordeum vulgare located from
nucleotide 3361 to nucleotide 2947.

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<220>
 <223> Modified gene based on *Hordeum vulgare* from
 nucleotide 1834 to nucleotide 1420 in *Zea mays*
 expression vector. *Zea mays* promoter from
 nucleotide 3271 to nucleotide 1834.

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 <211> 5115
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<220>

<223> Gene from *Hordeum vulgare* from nucleotide 1343 to
 nucleotide 1757 in *Zea mays* expression vector.
Zea mays promoter from nucleotide 43 to nucleotide
 1342.

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<211> 5392

<212> DNA

<213> Artificial Sequence

<220>

<223> Gene from Glycine max from nucleotide 2199 to
nucleotide 2675 in Zea mays expression vector.
Zea mays promoter from nucleotide 676 to
nucleotide 2198.

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<210> 7

<211> 5173

<212> DNA

<213> Artificial Sequence

<220>

<223> Gene from *Hordeum vulgare* from nucleotide 2199 to nucleotide 2450 in a *Zea mays* expression vector.

Zea mays promoter from nucleotide 676 to nucleotide 2198.

<400> 7

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<211> 54
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer designed based upon the alpha hordothionin sequence from Hordeum vulgare to amplify the gene and to introduce a NcoI site at the start (ATG) codon and a BamHI site after the stop codon of the thionin coding sequence to facilitate cloning.

 <400> 8
 agtataagta aacacaccat cacacccttg aggcccttgc tggtaggcat ggtg 54

 <210> 9
 <211> 55
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer designed based upon the alpha hordothionin sequence of Hordeum vulgare to amplify the gene and to introduce a NcoI site at the start (ATG) codon and a BamHI site after the stop codon of the thionin coding sequence to facilitate cloning.

 <400> 9
 cctcacatcc cttagtcct aagttcgacg tcgggccctc tagtcgacgg atcca 55

 <210> 10
 <211> 35
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer designed for single stranded DNA site-directed mutagenesis to introduce into the native Hordeum vulgare alpha hordothionin gene 12 codons for lysine, based on the peptide structure of hordothionin 12.

 <400> 10
 agcggaaaat gcccgaaagg cttccccaaa ttggc 35

 <210> 11
 <211> 45
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Primer designed for single stranded DNA site-directed mutagenesis to introduce into the native Hordeum vulgare alpha hordothionin gene 12 codons for lysine, based on the peptide structure of hordothionin 12.

 <400> 11
 tgcgcaggcg tctgcaagtg taagctgact agtagcgga aatgc 45

 <210> 12
 <211> 50

<212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer designed for single stranded DNA
 site-directed mutagenesis to introduce into the
 native Hordeum vulgare alpha hordothionin gene 12
 codons for lysine, based on the peptide structure
 of hordothionin 12.

<400> 12
 tacaaccttt gcaaagtcaa aggcgccaag aagctttgcg caggcgtctg 50

<210> 13
 <211> 50
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer designed for single stranded DNA
 site-directed mutagenesis to introduce into the
 native Hordeum vulgare alpha hordothionin gene 12
 codons for lysine, based on the peptide structure
 of hordothionin 12.

<400> 13
 gcaagagttg ctgcaagagt accctgggaa ggaagtgcta caacctttgc 50

<210> 14
 <211> 609
 <212> DNA
 <213> Pisum sativum

<220>
 <221> CDS
 <222> (18)...(410)

<400> 14
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 Met Ala Ser Val Lys Leu Ala Ser Leu Met Val
 1 5 10

ttg ttt gcc aca tta ggt atg ttc ctg aca aaa aac gta gga gca gca 98
 Leu Phe Ala Thr Leu Gly Met Phe Leu Thr Lys Asn Val Gly Ala Ala
 15 20 25

agc tgc aat ggg gtt tgt tct cca ttt gag atg cca cca tgt ggc tct 146
 Ser Cys Asn Gly Val Cys Ser Pro Phe Glu Met Pro Pro Cys Gly Ser
 30 35 40

tca gcc tgt cga tgt atc cct gtt ggt cta gtt gtt ggt tac tgc aga 194
 Ser Ala Cys Arg Cys Ile Pro Val Gly Leu Val Val Gly Tyr Cys Arg
 45 50 55

cat cca tct gga gtt ttc ttg agg acg aat gat gaa cac cct aac tta 242
 His Pro Ser Gly Val Phe Leu Arg Thr Asn Asp Glu His Pro Asn Leu
 60 65 70 75

tgt gag tct gat gcc gat tgt agg aag aaa gga agt ggt aac ttt tgc 290
 Cys Glu Ser Asp Ala Asp Cys Arg Lys Lys Gly Ser Gly Asn Phe Cys
 80 85 90

gcc tct gca atg cag atg ccc tgc ccc tgc gcg ggg ctg cag ggc ttg Ala Ser Ala Met Gln Met Pro Cys Pro Cys Ala Gly Leu Gln Gly Leu 20 25 30	332
tac ggc gct ggc gcc ggc ctg acg acg atg atg ggc gcc ggc ggg ctg Tyr Gly Ala Gly Ala Gly Leu Thr Thr Met Met Gly Ala Gly Gly Leu 35 40 45	380
tac ccc tac gcg gag tac ctg agg cag ccg cag tgc agc ccg ctg gcg Tyr Pro Tyr Ala Glu Tyr Leu Arg Gln Pro Gln Cys Ser Pro Leu Ala 50 55 60 65	428
gcg gcg ccc tac tac gcc ggg tgt ggg cag acg agc gcc atg tac cag Ala Ala Pro Tyr Tyr Ala Gly Cys Gly Gln Thr Ser Ala Met Tyr Gln 70 75 80	476
ccg ctc cgg caa cag tgc tgc cag cag cag atg agg atg atg gac gtg Pro Leu Arg Gln Gln Cys Cys Gln Gln Gln Met Arg Met Met Asp Val 85 90 95	524
cag tcc gtc gcg cag cag ctg cag atg atg atg cag ctt gag cgt gcc Gln Ser Val Ala Gln Gln Leu Gln Met Met Met Gln Leu Glu Arg Ala 100 105 110	572
gct gcc gcc agc agc agc ctg tac gag cca gct ctg atg cag cag cag Ala Ala Ala Ser Ser Ser Leu Tyr Glu Pro Ala Leu Met Gln Gln Gln 115 120 125	620
cag cag ctg ctg gca gtc cag ggt ctc aac ccc atg gcc atg atg atg Gln Gln Leu Leu Ala Val Gln Gly Leu Asn Pro Met Ala Met Met Met 130 135 140 145	668
gcg cag aac atg ccg gcc atg ggt gga ctc tac cag tac cag tac cag Ala Gln Asn Met Pro Ala Met Gly Gly Leu Tyr Gln Tyr Gln Tyr Gln 150 155 160	716
ctg ccc agc tac cgc acc aac ccc tgt ggc gtc tcc gct gcc att ccg Leu Pro Ser Tyr Arg Thr Asn Pro Cys Gly Val Ser Ala Ala Ile Pro 165 170 175	764
ccc tac tac tga ttcatgatat ttgggaaatc tcctctatcc atccctctct Pro Tyr Tyr * 180	816
atctatatat gtaataatgc agtaagacga cacacattat catgtgtggt atgaccaata atatatgcat cataataaag ttttggtttt aaagaattat cggacgcttg atatctatga tgctggataa atcaaaaactt ctcatataaa ttgtaaatat ttcaaaatct ctatttaggc tccaatggag agcatatggg tagagtagta tatatgcttg aaataactaac aactagcaaa gtgcggggcac gttgctacat gctcatttat gctcgagcat ggagtataaa acataaagat atatatgttc cattggcctg gtaaacgctg gatataggtt taaagccaac aactcatggt tcgaatcccc atttatatat aatccataat tttagcgctt tttaccattt aaattttgga gtaa	876 936 996 1056 1116 1176 1236 1240
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35	40	45
Leu Tyr Pro Tyr Ala Glu Tyr	Leu Arg Gln Pro Gln Cys Ser Pro Leu	
50	55	60
Ala Ala Ala Pro Tyr Tyr Ala Gly	Cys Gly Gln Thr Ser Ala Met Tyr	
65	70	75
Gln Pro Leu Arg Gln Gln Cys Cys	Gln Gln Met Arg Met Met Asp	
85	90	95
Val Gln Ser Val Ala Gln Gln Leu	Gln Met Met Met Gln Leu Glu Arg	
100	105	110
Ala Ala Ala Ala Ser Ser Ser Leu	Tyr Glu Pro Ala Leu Met Gln Gln	
115	120	125
Gln Gln Gln Leu Leu Ala Val Gln	Gly Leu Asn Pro Met Ala Met Met	
130	135	140
Met Ala Gln Asn Met Pro Ala Met	Gly Gly Leu Tyr Gln Tyr Gln Tyr	
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Gln Leu Pro Ser Tyr Arg Thr Asn	Pro Cys Gly Val Ser Ala Ala Ile	
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Pro Pro Tyr Tyr		
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ctttacttga	ggaaaaggaa	acaaatatga	tgtggccatc	acattctcaa	taacaatgac	180
catgtgcgca	atgacatacc	atcatatttg	atatcataaa	aataaattta	ttatcaaagt	240
aaacatatag	ttcatatatc	agatattaaa	gtgataagaa	caaataattac	attttatctt	300
atataaaatg	acgaaaaagg	tacgagttga	aaaggagtc	aaccctttt	ttatagcttg	360
ttcggttget	tggtctcttc	ggctagcgag	gtggtagaat	gtgagagtgt	tgcgcgtgga	420
ttcccgctcg	agtgttctta	ggtgatttct	cacggcccat	ctgtgatata	gcgactcata	480
tgtggtgtaa	tagcccatg	ggagaagggg	agagatatag	atctacgtga	tttgacgtg	540
atgcacgacg	aacgaaactg	gtggtttaaa	gtagtagagg	tttgtcatta	gaggtgtaaa	600
tggtacatat	attatccgtt	catattcgaa	tttgatccgt	ataagagggc	taagatctaa	660
tccgtataca	agtccaagta	ttaagtatcc	gatccatata	ggatctttat	ccgtatccgt	720
atactcaaaa	tttgatgttt	aagattttta	tatatattta	aactttatag	gaactcgata	780
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ccgttccgat	atttgtcatg	ctttgctaaa	aataccttta	caaggcatct	tgtgcagatt	900
atatattaat	ctgaaatcag	ttagagaagc	ctacaaattt	gaccaaatgc	cgagtcatcc	960
ggcttatccc	ctttccaact	ttcagttctg	caagcgccag	aaatcgtttt	tcactctacat	1020
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gcctcagtcg	cacatatcta	ctatactata	ctctaggaag	caaggacacc	accgcc atg	1139
				Met		
				1		

gca gcc aag atg ctt gca ttg ttc gct ctc cta gct ctt tgt gca agc	1187
Ala Ala Lys Met Leu Ala Leu Phe Ala Leu Leu Ala Leu Cys Ala Ser	
5 10 15	

gcc act agt gcg acc cat att cca ggg cac ttg cca cca gtc atg cca	1235
Ala Thr Ser Ala Thr His Ile Pro Gly His Leu Pro Pro Val Met Pro	
20 25 30	

ttg ggt acc atg aac cca tgc atg cag tac tgc atg atg caa cag ggg Leu Gly Thr Met Asn Pro Cys Met Gln Tyr Cys Met Met Gln Gln Gly 35 40 45	1283
ctt gcc agc ttg atg gcg tgt ccg tcc ctg atg ctg cag caa ctg ttg Leu Ala Ser Leu Met Ala Cys Pro Ser Leu Met Leu Gln Gln Leu Leu 50 55 60 65	1331
gcc tta ccg ctt cag acg atg cca gtg atg atg cca cag atg atg acg Ala Leu Pro Leu Gln Thr Met Pro Val Met Met Pro Gln Met Met Thr 70 75 80	1379
cct aac atg atg tca cca ttg atg atg ccg agc atg atg tca cca atg Pro Asn Met Met Ser Pro Leu Met Met Pro Ser Met Met Ser Pro Met 85 90 95	1427
gtc ttg ccg agc atg atg tcg caa ata atg atg cca caa tgt cac tgc Val Leu Pro Ser Met Met Ser Gln Ile Met Met Pro Gln Cys His Cys 100 105 110	1475
gac gcc gtc tcg cag att atg ctg caa cag cag tta cca ttc atg ttc Asp Ala Val Ser Gln Ile Met Leu Gln Gln Gln Leu Pro Phe Met Phe 115 120 125	1523
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gtt ggt gct gca ttc tag atagaaatat ttgtgttgta tcgaataatg Val Gly Ala Ala Phe * 150	1619
agttgacatg ccacgcgtg tgactcatta ttaacaataa aacaagtttc ctcttattat ctttttatat ctctccctat ccattttttgc aaagccatt atcctttact ccctaagtcc caatatattt tagaccttaa attgtatgtc tatattcaaa agaatgacaa taaatctaga catatatata aaacacatac attaatgatt gtatgaatct attaaaatgc taaaacgact aatattatgg gacggaggga gtactttatt agtagattac attgttattt tctctattcc aaatataagt ctgggttttc aatcaatcaa tatatattac catgtccaaa cattttgaat tatatatcta ggtgcagcat ccgtgcacga tcgtaaaaga agcagtcacg gtgttggtcc caaaaaactaa tcgtccgttg tcggtcacct ataaagattc atgaagagaa ccaaaataag gcaatataat taatgtaata tgactcctcc ttttgaatta cttaggaata acataagcaa acaaaaaaag gagaagatca aggtaaataa aggcattttg tgagaaaaca tggaagcata agaatgcata agtaatgatt tgtgtctctt tatatttttt ttattcacgt gaatttacat agataccatc ggatgttcga tggtaataca atgatgcctt agctccgaga gcttcgaatg atgagcgatt taaaaatact cctatcaatt gttcgaaagt tctttgtctc atgcatgggc aatgtacctc tatttatagg gacggtgcga cgtacaaatt tgtataaaat tatattttta ttcccaaatac ctatgcatat gtgtcgggga ccataattag ggtaccctc aaggctccta attctcagct ggtaacccca tcagcataaa gctgcaaagg cct	1679 1739 1799 1859 1919 1979 2039 2099 2159 2219 2279 2339 2399 2459 2519 2562
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50		55		60											
Leu	Ala	Leu	Pro	Leu	Gln	Thr	Met	Pro	Val	Met	Met	Pro	Gln	Met	Met
65				70					75						80
Thr	Pro	Asn	Met	Met	Ser	Pro	Leu	Met	Met	Pro	Ser	Met	Met	Ser	Pro
				85					90					95	
Met	Val	Leu	Pro	Ser	Met	Met	Ser	Gln	Ile	Met	Met	Pro	Gln	Cys	His
			100					105					110		
Cys	Asp	Ala	Val	Ser	Gln	Ile	Met	Leu	Gln	Gln	Gln	Leu	Pro	Phe	Met
		115					120					125			
Phe	Asn	Pro	Met	Ala	Met	Thr	Ile	Pro	Pro	Met	Phe	Leu	Gln	Gln	Pro
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Ala Tyr Thr Ser Lys Ile Phe Ala Leu Phe Ala Leu Ile Ala Leu Ser	
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gca agt gcc act act gca atc acc act atg cag tat ttc cca cca aca	152
Ala Ser Ala Thr Thr Ala Ile Thr Thr Met Gln Tyr Phe Pro Pro Thr	
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tta gcc atg ggc acc atg gat ccg tgt agg cag tac atg atg caa acg	200
Leu Ala Met Gly Thr Met Asp Pro Cys Arg Gln Tyr Met Met Gln Thr	
35 40 45 50	
ttg ggc atg ggt agc tcc aca gcc atg ttc atg tcg cag cca atg gcg	248
Leu Gly Met Gly Ser Ser Thr Ala Met Phe Met Ser Gln Pro Met Ala	
55 60 65	
ctc ctg cag cag caa tgt tgc atg cag cta caa ggc atg atg cct cag	296
Leu Leu Gln Gln Gln Cys Cys Met Gln Leu Gln Gly Met Met Pro Gln	
70 75 80	
tgc cac tgt ggc acc agt tgc cag atg atg cag agc atg caa caa gtt	344
Cys His Cys Gly Thr Ser Cys Gln Met Met Gln Ser Met Gln Gln Val	
85 90 95	
att tgt gct gga ctc ggg cag cag cag atg atg aag atg gcg atg cag	392
Ile Cys Ala Gly Leu Gly Gln Gln Gln Met Met Lys Met Ala Met Gln	
100 105 110	
atg cca tac atg tgc aac atg gcc cct gtc aac ttc caa ctc tct tcc	440
Met Pro Tyr Met Cys Asn Met Ala Pro Val Asn Phe Gln Leu Ser Ser	
115 120 125 130	
tgt ggt tgt tgt tga tcaaacgttg gttacatgta ctctagtaat aaggtgttgc	495
Cys Gly Cys Cys *	

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<213> Oryza sativa

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35 40 45
Gln Thr Leu Gly Met Gly Ser Ser Thr Ala Met Phe Met Ser Gln Pro
50 55 60
Met Ala Leu Leu Gln Gln Cys Cys Met Gln Leu Gln Gly Met Met
65 70 75 80
Pro Gln Cys His Cys Gly Thr Ser Cys Gln Met Met Gln Ser Met Gln
85 90 95
Gln Val Ile Cys Ala Gly Leu Gly Gln Gln Gln Met Met Lys Met Ala
100 105 110
Met Gln Met Pro Tyr Met Cys Asn Met Ala Pro Val Asn Phe Gln Leu
115 120 125
Ser Ser Cys Gly Cys Cys
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<212> PRT
<213> Triticum aestivum

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Leu Thr Ser Gly Leu Ser Cys Pro Lys Asp Phe Pro Lys
35 40 45